CVBIO.008CP1 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Stegmann, Thomas (as amended)
Appl. No.	:	10/649,480
Filed	:	August 27, 2003
For	:	METHOD OF PRODUCING BIOLOGICALLY ACTIVE HUMAN ACIDIC FIBROBLAST GROWTH FACTOR AND ITS USE IN PROMOTING ANGIOGENESIS
Examiner	:	Li, Bao Q
Group Art Unit	:	1648

DECLARATION OF INVENTORSHIP UNDER In re Katz, 687 F. 2d 450, 215 U.S.P.Q. 14 (CCPA, 1982)

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, Thomas Stegmann, declare as follows:

1. I am the sole inventor of the claims of the above-identified patent application as amended in the accompanying Amendment, and am responsible for the inventive concepts disclosed therein.

2. I, am also one of the co-authors of the publication: B. Schumacher, MD; P. Pecher, MD; B.U. von Specht, MD; Th. Stegmann, MD, (1998) "Induction of Neoangiogenesis in Ischemic Myocardium by Human Growth Factors" Circulation vol. 97, pages 645-650, wherein significant aspects of the invention are described.

3. The following co-authors of the publication contributed to the study presented in the publication as follows, in terms of (a) the position held at the time of the study described in the publication; and (b) contribution to the work:

B. Schumacher, MD, **(staff member & senior assistant in Department of Thoracic & Cardiovasular Surgery)**

Appl. No. : **10/649,480**
Filed : **August 27, 2003**

- assisted me during the coronary artery bypass graft (CABG) operations when intramyocardial FGF-1 injection was performed under my direct supervision
- performed collection and pre-evaluation of patient data under my supervision
- screened patients for the trial under my supervision
- collected data for the publication under my supervision

Peter Pecher, MD, **(staff member as non-certified cardiovascular surgeon)**

- collected patient data during the study
- provided surgical assistance in the operating room during the coronary artery bypass graft (CABG) operations while I performed the intramyocardial injection of FGF-1

B.U. von Specht, MD, **(employee of surgical research laboratory in Freiburg, Germany)**

- prepared FGF-1 used in the study following my instructions

4. The three co-authors listed above were involved only with providing assistance during the surgical procedures, screening patients for the study and preparation of the FGF-1 used in the study according to my directions. All three co-authors worked under my direct guidance and direction. They were not the inventors of the subject matter described in the patent application, but were listed as co-authors in order to receive credit for working on the project, as is the custom in scientific research programs.

5. For the reasons presented above, only myself, Thomas Stegmann, MD, who is listed as inventor on the above-referenced patent application is the true inventor of the subject matter claimed in the above-referenced application.

6. As a person signing below, I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States codes and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: August 22, 2005

By: ______________________
Thomas Stegmann, MD

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CVGENG.007A

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Stegmann, T.	) Group Art Unit 1651
Appl. No.	:	09/358,780	)
Filed	:	July 22, 1999	)
For	:	**INDUCTION OF NEOANGIOGENESIS IN ISCHEMIC MYOCARDIUM**	)
Examiner	:	Patten, P.	

SECOND DECLARATION UNDER 37 C.F.R. § 1.131

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

I Dr. Thomas Stegmann, declare as follows:

1. I am the inventor of the claims of the above-identified patent application and inventor of the subject matter described and claimed therein.

2. Prior to November 4, 1997, I had completed my invention as described and claimed in the above-referenced application in the United States or in a NAFTA or WTO country at a date prior to the date of publication of the reference: U.S. patent No. 6,045,565, filed November 2, 1998 which claims priority to Provisional application No. 60/064,210, filed November 4, 1997.

3. In the reference: Schumacher, et al. (February 24, 1998) "Induction of Neoangiogenesis in Ischemic Myocardium by Human Growth Factors" Circulation vol. 97 (7), pages 645-650, significant concepts of the invention are described, e.g. injection of human growth factor FGF-1 close to the vessels into ischemic tissue to induce neoangiogenesis and revascularization in

human subjects. The disclosure contained in the reference therefore implies a completion of the principal aspects of the invention and reduction to practice. The date of invention with respect to those concepts must necessarily precede the date the reference was submitted for publication, January 9, 1997. This reference was submitted with the Information Disclosure Statement of March 9, 2000 and is attached hereto as Exhibit A for the convenience of the Examiner.

4. However, even before the time of the Schumacher, et al. publication (Exhibit A), I had conceived the pharmaceutical composition of FGF-1 and physiologic glue for the purpose of injecting an amount of the composition into the ischemic myocardium at or near at least one predetermined site of coronary artery stenosis to induce local neoangiogenesis in connection with Claim 1 of the above-captioned patent application and was diligent in reduction to practice as shown by the Final Study Report for the FGF-1 Study (Exhibit B).

5. In the attached Final Study Report for the FGF-1 Study (Exhibit B) performed by myself, the injection of human growth factor FGF-1 with fibrin glue close to the vessels into ischemic tissue to induce neoangiogenesis and revascularization in human subjects is described. See "Methodology," page 4; "Test Product," page 5; "Conclusion," page 6; and "Treatments," page 9.

6. My method of revascularizing a region of ischemic myocardium using FGF-1 therefore antedates the November 4, 1997 filing date of Provisional Application No. 60/064,210, which discloses an increase in blood circulation to the myocardium by patent holes or injection into the myocardium with and without the use of angiogenic substances such as FGF-1.

7. Even as to those aspects of the invention that we conceived but had not reduced to practice at the reference date, we have worked diligently to achieve a constructive reduction to practice by preparing a patent application disclosing our invention in its entirety and filing that application on July 24, 1998 as Provisional application No. 60/093,962, six (6) months after the date of publication of the Schumacher et al. reference on February 24, 1998. The activity of drafting and filing a patent application demonstrates diligence from prior to the publication

of the cited reference on February 24, 1998, to a constructive reduction to practice July 24, 1998.

8. As a person signing below, I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States codes and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: May 05, 2002

By: _____
Thomas Stegmann, M.D.

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EXHIBIT A

Circulation 97(7):640-650
Feb. 24, 1998

submit c IDS of March 9, 2000

Clinical Investigation and Reports

Induction of Neoangiogenesis in Ischemic Myocardium by Human Growth Factors

First Clinical Results of a New Treatment of Coronary Heart Disease

B. Schumacher, MD; P. Pecher, MD; B.U. von Specht, MD; Th. Stegmann, MD

Background—The present article is a report of our animal experiments and also of the first clinical results of a new treatment for coronary heart disease using the human growth factor FGF-I (basic fibroblast growth factor) to induce neoangiogenesis in the ischemic myocardium.

Methods and Results—FGF-I was obtained from strains of *Escherichia coli* by genetic engineering, then isolated and highly purified. Several series of animal experiments demonstrated the apathogenic action and neoangiogenic potency of this factor. After successful conclusion of the animal experiments, it was used clinically for the first time. FGF-I (0.01 mg/kg body weight) was injected close to the vessels after the completion of internal mammary artery (IMA)/left anterior descending coronary artery (LAD) anastomosis in 20 patients with three-vessel coronary disease. All the patients had additional peripheral stenoses of the LAD or one of its diagonal branches. Twelve weeks later, the IMA bypasses were selectively imaged by intra-arterial digital subtraction angiography and quantitatively evaluated. In all the animal experiments, the development of new vessels in the ischemic myocardium could be demonstrated angiographically. The formation of capillaries could also be demonstrated in humans and was found in all cases around the site of injection. A capillary network sprouting from the proximal part of the coronary artery could be shown to have bypassed the stenoses and rejoined the distal parts of the vessel.

Conclusions—We believe that the use of FGF-I for myocardial revascularization is in principle a new concept and that it may be particularly suitable for patients with additional peripheral stenoses that cannot be revascularized surgically. (*Circulation*. 1998;97:645–650.)

Key Words: growth substances ■ angiogenesis ■ coronary disease

For the cardiac surgeon who is attempting to treat CHD, the use of sections of autologous blood vessels as bypass material is subject to severe limitations. Autologous arterial conduits are in short supply, and segments of the saphenous vein do not remain patent for very long.[1,2] Furthermore, "complete" revascularization is limited if diffuse coronary arteriosclerosis is present and extensive, especially if there are additional peripheral stenoses.

See p 628

In the search for alternative and/or additional treatment for improving the long-term prognosis, especially in diffuse CHD, attention has recently been directed toward natural angiogenesis.[3-9] Growth factors, especially FGF-I, have recently become of major importance because they can induce angiogenesis.[8,10-12]

Gimenez-Gallego et al[13] succeeded in elucidating the biochemical structure of FGF-I in 1985. Jaye et al[14] isolated human FGF-I from brain tissue in 1986. In 1991, Forough and coworkers[15] successfully used the technique of gene transfer to introduce the information for expressing human FGF-I into apathogenic *Escherichia coli*.

Our aim was to evaluate the information currently available on the biological effect of angiogenetic growth factors in animals and, if appropriate, to use human growth factor for the treatment of CHD. This involved (1) the production of human growth factor by genetic engineering, followed by its isolation, characterization, and purification; (2) using animal experiments to establish its angiogenetic potency and to exclude any possible pathogenic effect; and (3) using FGF-I clinically as an adjunct to coronary surgery and to demonstrate neoangiogenesis in the ischemic human myocardium.

Methods

Production and Purification of FGF-I

The production and purification of human FGF-I is a biochemically elaborate technique. The individual experimental steps have been reported elsewhere.[4,7]

Genetic engineering was used to produce human FGF-I from apathogenic strains of *E coli*, a plasmid containing the genetic information being introduced into the microorganisms.[15] These were kindly provided by Prof T. Maciag (Laboratory of Molecular Biology, American Red Cross, Rockville, Md). After production, FGF-I was eluted by heparin sepharose column chromatography, and several elution fractions were collected and purified by dialysis. Positive protein elution fractions were identified in the BIO-RAD assay[7] by SDS-PAGE,[16] and the biochemical isolation of FGF-I was confirmed by the Western blot method.[17] Further purification was obtained by HPLC.[18] The factors were lyophilized and stored at −32°C and diluted to 1 mL with NaCl solution containing 500 IU of heparin.

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Correspondence to B. Schumacher, MD, Klinik für Thorax-, Herz und Gefäßchirurgie, Klinikum Fulda, Pacelliallee 4, D-36043 Fulda, Germany.

Selected Abbreviations and Acronyms

CHD = coronary heart disease
EDP = electronic data processing
FGF = basic fibroblast growth factor
HPLC = high-pressure liquid chromatography
IMA = internal mammary artery
LAD = left anterior descending coronary artery

Chorioallantoic Membrane Assay

This established method, which provides a direct demonstration of the effect of growth factors on living tissue, was used to investigate the angiogenetic effect of FGF-I.[19,20] The growth of the allantoic systems can be directly observed by light microscopy. After incubation of 20 fertilized hen eggs for 13 days, the growth factor was applied to the membrane and covered with tissue culture coverslips. Four days later, the membrane was examined under the light microscope and directly compared with controls untreated with FGF-I or treated with heat-denatured FGF-I (70°C for 3 minutes).

Exclusion of the Pyrogenicity of FGF-I

Varying concentrations of FGF-I (0.01, 0.5, or 1.0 mg/kg body weight) were injected subcutaneously, intramuscularly, or intravenously into 27 New Zealand White rabbits, the solvent alone being used for an additional 13 controls. Thereafter, the rectal temperature was taken every half hour for 3 hours, hourly for the rest of the day, and every 8 hours for 12 days. A daily white cell count was also repeated for 12 days (see "Results"). In addition to this, the erythrocyte sedimentation rate and the C-reactive protein values were determined on the 3rd, 6th, 9th, and 12th days after the injection.

Confirmation of the Angiogenetic Potency of FGF-I in Animal Experiments

Supplementary to our earlier experiments,[9] the effect of FGF-I was also investigated in the ischemic hearts of inbred Lewis rats (a total of 275 animals, including 125 controls treated with heat-denatured FGF-I, 70°C for 3 minutes). The pericardium was opened via the abdominal wall and diaphragm, and two titanium clips were inserted at the apex of the left ventricle to induce myocardial ischemia. Growth factor (mean concentration of 10 μg) was then injected locally into the site. The coronary vessel system was imaged by aortic root angiography after 12 weeks and, finally, a specimen from the same myocardial region was evaluated histologically.

Clinical Use of FGF-I in Patients With CHD

This study was approved by the Medical Research Commission at the Phillips University of Marburg on August 10, 1993 (No. 47/93). This is the usual ethics commission for our hospital. Twenty patients without any history of infarction or cardiac surgery (14 men and 6 women; minimum age, 50 years) were subjected to an elective bypass operation for multivessel coronary heart disease. The growth factor was applied directly during the operation. As a control group, 20 patients who underwent the same procedure were given heat-denatured FGF-I (70°C for 3 minutes). The choice of treatment was completely random, the names being placed in sealed envelopes and selected in a blinded manner.

The details, nature, and aims of this procedure were explained beforehand to every patient who underwent the operation. In all cases, we received their fully informed consent. Both groups of patients were closely comparable with regard to clinical symptoms, accompanying disorders, cardiovascular risk factors, ventricular function, sex, and age. A comparable coronary morphology was found in both groups.

All patients had a further stenosis in the distal third of the LAD or at the origin of one of its branches in addition to a severe proximal stenosis. The mean ejection fraction of the left ventricle for all patients was 50%. The operative procedure for coronary revascularization with autologous grafts (an average per patient of 2 to 3 venous bypasses and 1 from the left IMA) was routinely performed. FGF-I (mean concentration, 0.01 mg/kg body weight) was injected into the myocardium, distal to the IMA/LAD anastomosis and close to the LAD, during the maintenance of the extracorporeal circulation and after completion of the distal anastomoses (Fig 1). In the control group, heat-denatured FGF-I was substituted for FGF-I. After 12 weeks, the IMA bypasses of all the patients were imaged selectively by transfemoral, intra-arterial, and digital subtraction angiography.

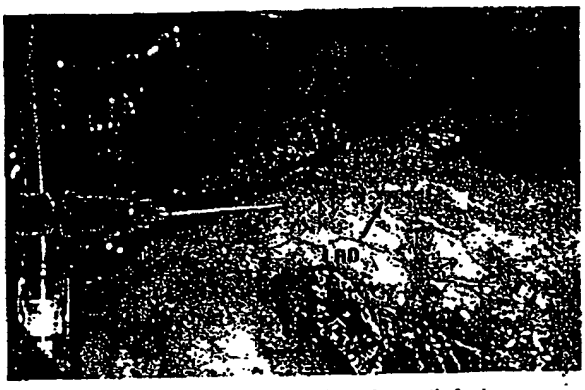


Figure 1. Intraoperative administration of growth factor.

Angiograms obtained in this way were evaluated by means of EDP-assisted digital gray-value analysis, a universally recognized and well-established technique for demonstrating capillary neoangiogenesis.[21–24] Sites of interest both with and without FGF-I (meaning heat-denatured FGF-I) were selected in the vessels filled with contrast medium and in regions of the myocardium distal to the IMA/LAD anastomosis. One hundred pixels were selected from each site of interest and analyzed digitally. Complete blackening of the x-ray films was rated with a gray value of 150, and areas without blackening of the film were allotted a zero value. During the first 5 postoperative days, separate laboratory checks in addition to the routine postoperative follow-up procedures were made twice daily, and the temperature checked three times a day.

Results

After separation, purification, and stabilization, we were able to isolate human FGF-I in all 40 bacterial cultures and demonstrate its high degree of purity. Fig 2 shows an HPLC profile of the growth factor after routine purification. The peak values at the beginning and end of the profile represent impurities that could be identified as *E coli* proteins. FGF-I could be further separated by fractionated collection, and the control HPLC (Fig 3) merely shows the peak value of this fraction on an otherwise even baseline.

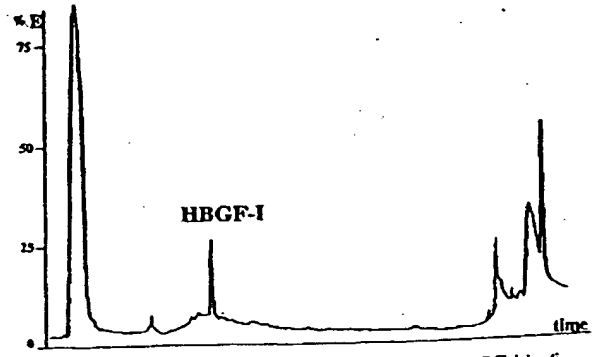


Figure 2. HPLC profile before high purification. HBGF-I indicates human FGF-I; %E, extinction.

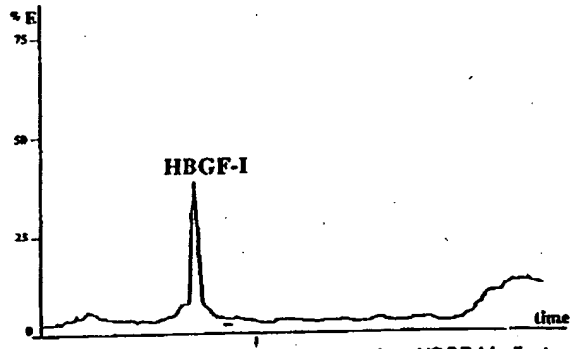


Figure 3. HPLC profile after high purification. HBGF-I indicates human FGF-I; %E, extinction.

In the chorioallantoic membrane assay, the angiogenetic potency of FGF-I could be demonstrated in vivo. As early as 4 days after application of the factor, the vascular structure of the membrane was completely altered. Emanating radially from the site of application, an unequivocal growth of new vessels from the original host vessels had grown out into the periphery (Fig 4A). These structures were completely absent from the control group, and a normally developed reticular vascular pattern could be discerned (Fig 4B).

Pyrogenic effects of the human growth factor produced in this way could be definitively ruled out in the animal model. There was no significant rise of body temperature when checked at short intervals and no trace of an inflammatory reaction in comparison with the control group (n=13) in any of the 27 test animals during the period of observation. This result was independent of the concentration and the route of administration (intravenous, subcutaneous, or intramuscular) of the factor.

Earlier investigations into the application of FGF-I to the nonischemic rat heart made it possible to demonstrate neoangiogenesis both histologically and angiographically after 9 weeks in 11 of 12 test animals after the implantation of a tissue bridge pretreated with growth factor between the heart and thoracic aorta. In the control group without FGF-I (n=6), no signs of induced neoangiogenesis could be found.[47]

Unequivocal proof of induced neoangiogenesis was also found in the ischemic rat heart. In the test animals, in which myocardial ischemia had previously been induced with titanium clips and growth factor had subsequently been injected into the myocardium, a manifest accumulation of contrast medium was shown by aortic angiography at the site of the FGF-I injection 12 weeks later (Fig 5A), whereas such an accumulation of contrast medium did not appear in any of the control animals (Fig 5B). Histological examination of the myocardium revealed a threefold increase in the capillary density per square millimeter around the site of the FGF-I injection.

When the growth factor FGF-I was used clinically for the first time on the human heart, neoangiogenesis together with the development of a normal vascular appearance could be demonstrated angiographically, exactly as in the earlier animal experiments.[47] Selective imaging of the IMA bypasses by intra-arterial digital subtraction angiography confirmed the following result in all 20 patients: at the site of injection and in the distal areas supplied by the LAD, a pronounced accumulation of contrast

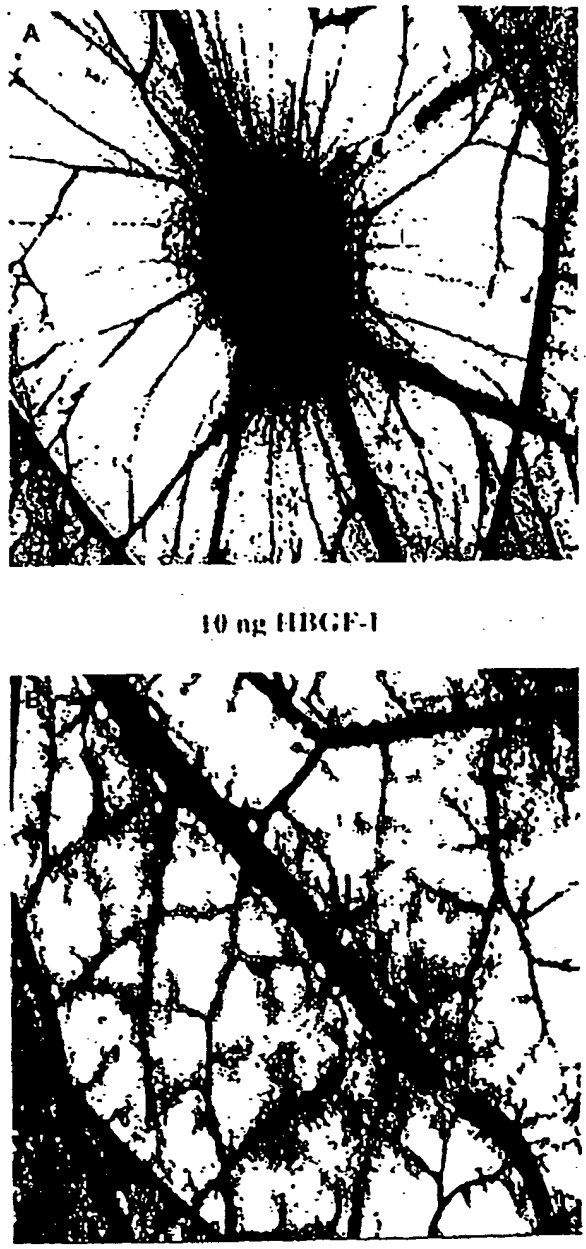


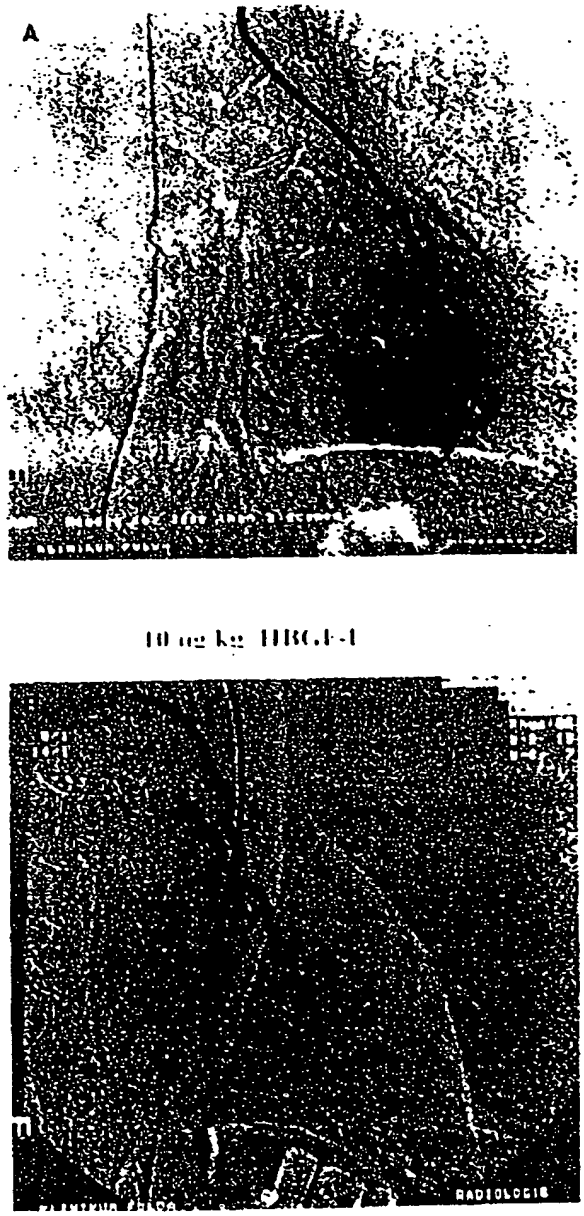
Figure 4. A, Chorioallantoic membrane assay with application of the growth factor. B, Chorioallantoic membrane assay of the control group. HBGF-I indicates human FGF-I.

medium extended peripherally around the artery for ≈3 to 4 cm, distal to the IMA/LAD anastomosis (Fig 6A). In the control angiograms of patients to whom only heat-denatured FGF-I had been given, the IMA/LAD anastomosis was also recognizable, but the accumulation of contrast medium described above was absent (Fig 6B). The angiograms of both the treated and control groups were recorded at a rate of four images per second, and these show

10 μg HBGF-1

without HBGF-1

Figure 5. A, Administration of the growth factor in ischemic rat heart with a clearly discernible accumulation of contrast medium at the site of injection. B, No discernible accumulation of contrast medium in the control group. HBGF-1 indicates human FGF-I.



10 ng/kg HBGF-1

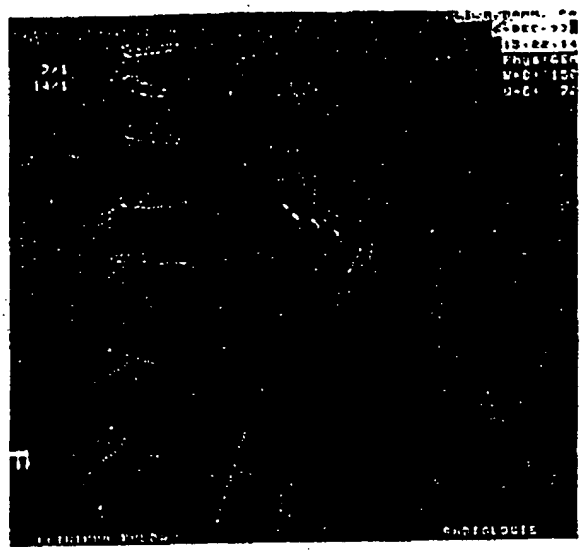
without HBGF-1

Figure 6. A, Angiography after injection of the growth factor into the human heart shows a pronounced accumulation of contrast medium compared with the control group. B, Angiography in the control group does not show any increased accumulation of contrast medium around the IMA/LAD anastomosis. HBGF-1 indicates human FGF-I.

comparable distances between the beginning of the injection and visualization of the medium.

At the site of injection of the FGF-I, a capillary network could be seen sprouting out from the coronary artery into the myocardium. This enabled retrograde imaging of a stenosed diagonal branch to be performed (Fig 7A). Such "neocapillary vessels" can also provide a collateral circulation around additional distal stenoses of the LAD (Fig 7B) and bring about retrograde filling of a short segment of the artery distal to the stenosis. In none of the angiograms of the treated patients taken 12 weeks after the operation were any new stenoses of the LAD detectable.

The results of EDP-assisted digital gray value analysis for quantification of the neoangiogenesis (Fig 8) gave a mean gray value of 124 for the vessels. The control myocardium reached



10 µg/kg HBGF-1

10 µg/kg HBGF-1

Figure 7. A, Collateralization of stenoses (arrow): a diagonal branch occluded just distal to its origin is filled through the newly grown capillaries. B, Collateralization of stenoses (arrow) by newly grown capillaries: the peripherally stenosed LAD is filled through these vessels. HBGF-1 indicates human FGF-1.

a gray value of only 20, and that of the myocardium injected with FGF-1 gave a value of 59 (Fig 8).

Discussion

Normal capillaries have a cell population with a low turnover rate of months or years. On occasion, however, a high turnover rate of this cell population is possible even under physiological

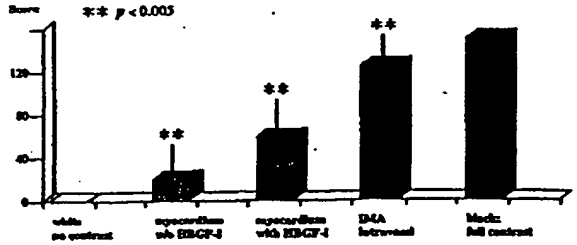


Figure 8. Quantitative gray value analysis of contrast medium accumulation in the angiography shows a twofold to threefold increase in the local blood flow at the site of injection. HBGF-1 indicates human FGF-1.

conditions, and this naturally leads to the rapid growth of new capillaries and other blood vessels. Such a physiological process occurs in the development of the placenta, in fetal growth, and in wound healing, as well in the formation of collaterals in response to tissue ischemia. "Angiogenetic growth factors," which are biochemically polypeptides, are essential for such processes as capillary growth or neoangiogenesis. These growth factors (for instance, the human heparin-binding FGF-1) bring about their effect by significantly increasing cell proliferation, differentiation, and migration via a high-affinity receptor system on the surfaces of the endothelial cells.[5,10–12]

During the last few years, several working groups have been able to establish indications for the effective use of growth factors to improve blood flow in the presence of tissue ischemia in animal experiments.[3,9,27] Yanagisawa-Miwa et al[9] succeeded in demonstrating a significant collateralization together with reduction in the size of the infarct after intracoronary administration of growth factor in rabbits. Baffour et al[3] also observed a significant formation of collaterals in ischemic extremities after growth factor administration in animals. Albes et al[27] produced a distinct improvement in the blood flow in ischemic tracheal segments implanted subcutaneously in rabbits by injecting growth factor–enriched fibrin glue locally.

After growth factor was injected into the ischemic rat heart,[4,7] we were able to observe induced neoangiogenesis and confirm it angiographically. We were also able to prove histologically that this neoangiogenesis brings about the development of new vascular structures with a three-layered vessel wall. Angiographic imaging confirmed that these are anatomically normal capillaries and other blood vessels.

The production of human FGF-1 by our molecular biological method has proved to be a complex but readily reproducible procedure. From the bacterial cultures, we are able to isolate the factor as a pure substance in sufficient quantities. By in vitro assay and as a result of extensive animal experiments, we were able to exclude the possible pyrogenic effects of FGF-1.

In earlier animal experiments,[4] we were able to demonstrate the proliferative and mitogenic effects of the growth factor on human saphenous vein endothelial cells. Endothelial cell cultures with added growth factor induced a confluent monolayer after only 5 to 9 days, whereas the monolayer was not complete before 7 to 11 days in the control group. In addition to determining the total cell count with a cell counter, we also confirmed this result by analyzing the rate of DNA synthesis by measuring the incorporation of ^{3}H-thymidine into the endothelial cell nuclei using the

method of Klagsbrun and Shing.[28] The cell proliferative potency of FGF-I could be further intensified by adding heparin, a glycosaminoglycan protecting the growth factor from inactivation by cellular enzymes and from heat and chemical denaturation.[29]

On the basis of these in vitro and in vivo experiments, we established for the first time the efficacy of FGF-I for the treatment of CHD, and were able to demonstrate that it can induce neoangiogenesis in situ in the ischemic human heart. This possibility has been widely discussed for many years but never before attempted.

A dense capillary network appeared around the site of injection of the factor in the myocardium of all our treated patients. This capillary network is a true de novo vascular system. Emerging from the proximal segment of the LAD, it sprouts out into the surrounding myocardium, bringing about a twofold to threefold increase in the local blood supply through these newly formed functional vessels. We were able to use the recognized physiological effects of FGF-I (as they occur in the repair mechanism of wound healing or in collateralization of ischemic tissue) to induce neoangiogenesis in the human ischemic heart.

We also consider that administration of FGF-I (produced in this way by genetic engineering), combined with operative myocardial revascularization, may well be an especially appropriate treatment for patients with additional peripheral stenoses that cannot be treated surgically.

In our opinion, neoangiogenesis induced by FGF-I opens up new possibilities for the treatment of ischemic myocardial disease. Furthermore, it could become a new therapeutic concept in the management of diffuse CHD after alternative methods of administration have also been developed. This method of inducing neoangiogenesis is also conceivable as a therapeutic option in other regions of the cardiovascular system in which arterial occlusion has led to ischemia.[30] However, before any such possibilities are realized, many more clinical investigations will have to be performed.

References

1. Kirklin JW, Subcommittee, and Task Force Members. Guidelines and indications for coronary artery bypass graft surgery: a report of the American College of Cardiology/American Heart Association Task Force on assessment of diagnostic and therapeutic cardiovascular procedures. *J Am Coll Cardiol.* 1991;17:543–589.
2. Lytle BW, Loop FD, Cosgrove DM, Ratey K, Taylor PC. Long-term (five to twelve years) serial studies of internal mammary artery and saphenous vein coronary bypass graft. *J Thorac Cardiovasc Surg.* 1985;89:248–258.
3. Baffour R, Berman J, Garb JL, Rhee SW, Kaufman J, Friedmann P. Enhanced angiogenesis and growth of collaterals by in vivo administration of recombinant basic fibroblast growth factor in a rabbit model of acute lower limb ischemia: dose-response effect of basic fibroblast growth factor. *J Vasc Surg.* 1992;16:181–191.
4. Fasol R, Schumacher B, Schlaudraff K, Hauenstein KH, Seitelberger R. Experimental use of a modified fibrin-glue to induce site-directed angiogenesis from the aorta to the heart. *J Thorac Cardiovasc Surg.* 1994;107:1432–1439.
5. Schlaudraff K, Schumacher B, Seitelberger R, v. Specht B-U, Schlosser V, Fasol R. An experimental study of in situ and in vivo growth of new arteries by the angiogenetic growth factor HBGF-I. *Thorac Cardiovasc Surg.* 1992;40:136.
6. Schlaudraff K, Schumacher B, Seitelberger R, v. Specht B-U, Schlosser V, Fasol R. Can you grow new arteries for CV surgeons? *J Cardiovasc Surg.* 1992;33:47.
7. Schlaudraff K, Schumacher B, v. Specht B-U, Seitelberger R, Schlosser V, Fasol R. Growth of new coronary vascular structures by angiogenetic growth factors *Eur J Cardiothorac Surg.* 1993;7:637–644.
8. Thompson JA, Anderson KD, Di Pietro JM, Zwiebel JA, Zametta M, Anderson WF, Maciag T. Site-directed neovessel formation in vivo. *Science.* 1988;241:1349–1352.
9. Yanagisawa-Miwa A, Uchida Y, Nakamura F, Tomaru T, Kido H, Kamijo T, Sugimoto T, Kaji K, Utsuyama M, Kurashima C, Ito H. Salvage of infarcted myocardium by angiogenic action of basic fibroblast growth factor. *Science.* 1992;257:1401–1402.
10. Dionne CA, Crumley G, Bellot F, Kaplow JM, Searfoss G, Ruta M, Burgess WH, Jaye M, Schlessinger J. Cloning and expression of two distinct high-affinity receptors cross-reacting with acidic and basic fibroblast growth factors. *EMBO J.* 1990;9:2685–2692.
11. Folkman J, Klagsbrun M. Angiogenetic factors. *Science.* 1987;235:442–447.
12. Friesel R, Burgess WH, Mehlman T, Maciag T. The characterization of the receptor for endothelial cell growth factor by covalent ligand attachment. *J Biol Chem.* 1986;261:7581–7584.
13. Gimenez-Gallego G, Rodkey J, Bennett C, Rios-Candelore M, DiSalvo J, Thomas K. Brain-derived acidic fibroblast growth factor: complete amino acid sequence and homologies. *Science.* 1985;230:1385–1388.
14. Jaye M, Howk R, Burgess W, Ricca G, Chiu IM, Ravera M, O'Brien S, Modi W, Maciag T, Drohan W. Human endothelial cell growth factor: cloning, nucleotide sequence and chromosome localization. *Science.* 1986;233:541–545.
15. Forough R, Engleka K, Thompson JA, Jackson A, Imamura T, Maciag T. Differential expression in *Escherichia coli* of the alpha and beta forms of heparin-binding acidic fibroblast growth factor 1: potential role of RNA secondary structure. *Biochem Biophys Acta.* 1991;1090:293–298.
16. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* 1970;227:680–685.
17. Bjerrum OJ, Heegaard NHH, eds. *Handbook of Immunoblotting of Proteins, Volume I: Technical Descriptions.* Boca Raton, Fla: CRC Press; 1988.
18. Gospodarowicz D, Cheng J, Lui GM, Baird A, Böhlent P. Isolation of brain fibroblast growth factor by heparin-sepharose affinity chromatography: identity with pituitary fibroblast growth factor. *Proc Natl Acad Sci U S A.* 1984;81:6963–6967.
19. Auerbach R, Kubai L, Knighton D, Folkman J. A simple procedure for the long-term cultivation of chicken embryo. *Dev Biol.* 1974;41:391–394.
20. Wilting J, Christ B, Bokeloh M. A modified chorioallantoic membrane (CAM) assay for qualitative and quantitative study of growth factors. *Anat Embryol.* 1991;183:259–271.
21. Wilson RF, Johnson MR, Marcus ML, Aylward PE, Skorton DJ, Collins S, White CW. The effect of coronary angioplasty on coronary flow reserve. *Circulation.* 1988;77:873–885.
22. Savchenko AP, Pomeranzev EV, Abugov SA, Blank ML. A videodensometric analysis of the characteristics of coronary blood flow using computed digital angiography in patients with ischemic heart disease. *Bull Vsesoiuznogo Kardiol Nauchn Tsentra AMN SSSR.* 1988;11:96–102.
23. Pijls NH, Bos HS, Uijen GJ, Van der Werf T. Is nonionic isotonic Iohexol the contrast agent of choice for quantitative myocardial videodensitometry? *Int J Card Imaging.* 1988;3:117–126.
24. Chang BL, Drury, JK, Meerbaum S, Fishbein MC, Whiting JS, Corday E. Enhanced myocardial washout and retrograde blood delivery with synchronized retroperfusion during acute myocardial ischemia. *J Am Coll Cardiol.* 1987;9:1091–1098.
25. Nishimura RA, Rogers PJ, Holmes DR JR, Gehring DG, Bove AA. Assessment of myocardial perfusion by videodensitometry in the canine model. *J Am Coll Cardiol.* 1987;9:891–897.
26. Schlaudraff K, von Specht BU, Kolvenbach H, Stegmann T, Schumacher B. Induktion neuer funktioneller Blutgefäße beim Menschen. *Langenbecks Arch Chir Chirurgisches Forum.* 1994;164–172.
27. Albes JM, Kletzner T, Kotzerke J, Thiedemann KU, Schäfers HJ, Borst HG. Improvement of tracheal autograft revascularisation by means of fibroblast growth factor. *Ann Thorac Surg.* 1994;57:444–449.
28. Klagsbrun M, Shing Y. Heparin affinity of anionic and cationic capillary endothelial cell growth factors: analysis of hypothalamus-derived growth factors and fibroblast growth factors *Proc Natl Acad Sci U S A.* 1985;82:805–809.
29. Rosengart TK, Johnson WV, Friesel RE, Clark R, Maciag T. Heparin protects heparin-binding growth factor-1 from proteolytic inactivation in vitro. *Biochem Biophys Res Comm.* 1988;152:432–440.
30. Ware A, Simons M. Angiogenesis in ischemic heart disease. *Nat Med.* 1997;3:158–164.

EXHIBIT B

QUINTILES

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MEMORANDUM

To:	Elizabeth Gordon	Copy:	Stefan Rettig (memo) Joachim A Schwarz (memo)
From:	Ludger Langer		
Date:	06 April 1999		
Subject:	FGF-I, Final Study Report		

Dear Elizabeth,

Please find attached the original final study report for the FGF-I study performed by Professor Stegmann, Fulda, Germany. I understand that you will take care of the distribution to the appropriate people.

Professor Stegmann is looking forward to "receive the final report" (which should be the one which would be sent officially to CVGE). He does not seem to have kept a copy of the report for his files when we provided the document to him as a co-author and asked for his signature.

Please call/e-mail in case of any questions.

With kind regards,

Title Page

"Induction of Local Neo-Angiogenesis in Cardiovascular Diseases by means of Growth Factors obtained by Genetic Engineering"

Medicinal product:	Human fibroblast growth factor FGF-I (acidic fibroblast growth factor)
Indication:	One-, two- or three-vessel coronary artery disease.
Design:	Double-blind, randomized, placebo-controlled, parallel, single center pilot study in patients with one-, two- or three-vessel coronary artery disease during coronary artery bypass grafting (CABG).
Sponsor:	Prof. Dr. med. Thomas Stegmann, Department of Thoracic and Cardiovascular Surgery, Fulda Medical Center, 36043 Fulda, Germany Phone Number: +49-661/84-5650, Fax Number: +49-661/84-5651 E-mail: skf-heart.stegmann@t-online.de
Protocol identification:	Date August 1, 1993
Development phase:	Phase I
Study initiation date (first patient enrolled):	August 15, 1993
Study completion date (last patient completed):	March 31, 1995
Principal investigators:	Prof. Dr. med. Thomas Stegmann, Department of Thoracic and Cardiovascular Surgery, Fulda Medical Center 36043 Fulda, Germany
Name of Company Signatory:	Dr. med. Joachim A. Schwarz, Dr. phil. nat. Ludger Langer, Quintiles GmbH, Schleussnerstrasse 42, D-63263 Neu-Isenburg Phone Number: +49-6102-296-0, Fax Number: +49-6102-296296, E-mail: jschwarz@qffm.quintiles.com, llanger@qffm.quintiles.com
Issue Date:	March 1999

Synopsis

Name of Company: CardioVascular Genetic Engineering Name of Active Ingredient: Human fibroblast-growth-factor-1 (FGF-I)	TABULAR FORMAT REFERING TO PART OF THE DOSSIER Volume: Page:	(For National Authority Use Only)
Title of Study: Induction of Local Neo-Angiogenesis in Cardiovascular Diseases by means of Growth Factors obtained by Genetic Engineering		
Investigator: Prof. Dr. med. Thomas Stegmann		
Study center: Department of Thoracic and Cardiovascular Surgery, Fulda Medical Center, Pacelliallee 4, 36043 Fulda, Germany		
Publication (reference): Induction of Neo-Angiogenesis in Ischemic Myocardium by Human Growth Factors - First Clinical Results of a New Treatment of Coronary Heart Disease B. Schumacher, MD; P. Pecher, MD; B.U. von Specht, MD; Th. Stegmann, MD, Circulation.1998; 97:645-650		
Study period (years): 1993 – 1995		Clinical Phase: I
Objectives: Induction of neo-angiogenesis in the ischemic myocardium in patients with 1-, 2- or 3-vessel coronary artery disease during coronary artery bypass grafting (CABG) (internal mammary artery (IMA bypass) / left anterior descending coronary artery (LAD) anastomosis)		
Methodology: Human Fibroblast Growth Factor I (FGF-I) / heat denaturated FGF-I (0,01 mg/kg body weight) was administered distal to the IMA/LAD anastomosis and close to the LAD after completion of internal mammary artery (IMA) / left anterior descending coronary artery (LAD) anastomosis. Twelve weeks later, the IMA bypasses were selectively imaged by intra-arterial digital subtraction angiography to quantitatively evaluate the efficacy of FGF-I by the formation of local neo-angiogenesis.		

Number of subjects (total and for each treatment): a total of 40 patients, 20 per treatment group was enrolled.
Diagnosis and criteria for inclusion: Patients with coronary 1-, 2- or 3-vessel disease affecting the LAD and its lateral branches during Coronary Artery Bypass Graft (CABG); > 50 years of age without past medical history of infarction; Ejection Fraction (EF): above 40%; cardiac catheter examination with coronary angiography: no longer than 6 months before surgery; revascularisation of LAD by means of IMA-bypass. No additional/simultaneous intervention (e.g. cardiac valve, carotid artery, or other) indicated. Exclusion criteria: Age: < 50 years or > 80 years; past medical history of myocardial infarction, revascularisation of LAD with venous bypass; cardiac catheter examination with coronary angiography: > 6 months preoperatively; EF of Left Ventricle (LV): < 40%.
Test product, dose, mode of administration, no batch available: Fibroblast Growth Factor I (FGF-I) was injected - 0,01 mg/kg body weight, 500IU heparin in physiological NaCl, 1 ml fibrin glue - into the myocardium close to the vessels surrounding the ischemic myocardium
Duration of treatment: single dose application

Reference therapy, dose, mode of administration, no batch available: heat-denaturated FGF-I (70°C for 3 minutes) – 0,01 mg/kg body weight, 500IU heparin in physiological NaCl, 1 ml fibrin glue - was injected into the myocardium close to the vessels surrounding the ischemic myocardium
Criteria for evaluation: Efficacy: Formation of new blood vessels assessed by quantitatively selective digital subtraction angiography (DSA) of the Internal mammary artery bypass and the area of interest (diagonal branch of LAD or peripheral LAD depending on pre-operative angiography results). Left ventricular function assessed by transthoracal echocardiography (TTE) Survival Safety Events fulfilling the definition of Serious Adverse Event(s) (SAE) Adverse Events related to study medication Standard routine laboratory assessments (not documented in CRF)
Statistical methods: The evaluation was performed using descriptive statistics only. In addition, an exploratory t-test was used to test for the difference in the area of interest between the two treatment groups.

SUMMARY – CONCLUSION

Efficacy results
Growth of new blood vessels could be demonstrated in the FGF-I group. Statistical analysis of Digital Subtractive Angiography (DSA) results of the area of interest revealed significant differences of gray values between the FGF-I group and the control group (59.2 in the FGF-I group versus 20.1 in the control group, $p < 0.001$). All patients were alive at month 12. There were no statistically significant differences between the echocardiography results of the FGF-I and control group.

Safety results
No adverse event or abnormal laboratory result was reported which was considered at least possibly related to study medication by the investigator. One patient was hospitalized for operation of a hernia inguinalis.

Conclusion
FGF-I was safely administered to 20 patients, heat denaturated FGF-I to another 20 patients. Growth of blood vessels could be demonstrated by comparison of the area of interest with the control group having received heat denaturated FGF-I.

Date of report January 1999

Investigational Plan

Due to the variety of clinical pictures associated with a reduction of organ or tissue perfusion, the collateralization of ischemic regions shall be improved by the use of angiogenetic growth factors. The targeted administration of human, angiogenetic factors may permit to induce "new" tissue growth at the sites of application. Thus, human, biological polypeptides could not only be used in vascular surgery, but also in heart and transplantation surgery in terms of additional bypass material. If the body reacts favorably to such factor applications, the induced development of endogenous tissue might ultimately lead to the formation of extensive collateral systems contributing considerably to the long-term prognosis of implants compared with autologous and heterologous materials. The aim of this experimental and clinical research project was to examine whether the human Fibroblast Growth Factor I (FGF-I), a member of the group of Heparin-Binding-Growth-Factors" (HBGF) in patients with coronary heart disease may induce the growth of new functional blood vessels.

The first clinical study of FGF-I was a double-blind, randomized, placebo-controlled (denaturated FGF-I), parallel, single center pilot study.

In the course of an elective aorto-coronary bypass surgery, the investigational medicinal product was administered by local intramyocardial / subepicardial injection in the area of inoperable stenoses / occlusions of the LAD and its lateral branches (e.g. LAD, D_1, D_2, D_3) after the completion of all anastomoses and before the termination of extracorporeal circulation (ECC).

During the course of the study no amendment was issued.

Study Objectives

The aim of the clinical study is to assess the ability of human growth factor FGF-I (one of Heparin-Binding-Growth-Factors - HBGF-I) administered locally to induce the growth of new functional blood vessels in patients with coronary heart disease.

Description of Study Design and Choice of Control Groups

This was a double-blind, randomized, placebo-controlled, parallel, single center pilot study in patients with three-vessel coronary artery disease during coronary artery bypass grafting (CABG).

As control group, 20 patients were given heat-denaturated (70°C for 3 minutes) FGF-I. The patients were allocated randomly to either FGF-I or heat-denaturated FGF-I by use of blinded coding envelopes ."Randomization Procedure": All participating patients were identified at study start. Patient names were put into sealed numbered envelopes, "mixed", and put one after in the other in the FGF-I group or the control group. The operating surgeon was not informed whether the active form of FGF-I or the inactivated one was injected.

For this pilot study a sample size calculation was not performed. It was decided that 20 patient treated with the investigational medicinal product would be adequate to demonstrate efficacy by neo-angiogenesis.

Subjects waiting for elective CABG were pre-selected on the basis of the available documentation in the patient files and the inclusion and exclusion criteria. Subjects were then contacted and asked whether they would be interested to take part in a clinical trial to assess the neo-angionetic potency of FGF-I.

The flow chart reflects the examinations performed during the study. In contrast to the original plan described in the IEC submission, only 1 DSA analysis was performed.

Flow Chart

	Pre-operation	CABG	12 weeks follow-up	12 months follow-up
History & IC	X			
Physical examination	X			
Coronary angiography	X			
Study medication		X		
Echocardiography (TTE)	X		X	X
selective digital subtraction angiography (DSA)			X	
Safety (SAEs and/or events associated to study drug)		X	X	X
Survival			X	X

After receipt of the patient's informed consent, a physical examination was performed. An angiogram would be performed in case the available angiogram was performed more than 6 months prior to the study. An echocardiographic assessment of the left ventricular ejection fraction had to prove an ejection fraction of at least 40%.

The CABG was performed in eligible patients using standard operation with HLM: mild hypothermia (28 - 30 C) and cold cardioplegic cardiac arrest (St. Thomas cardioplegic solution). Peripheral coronary anastomoses was performed prior to central anastomoses; administration of FGF-I (or denaturated factor) before the termination of ECC; FGF-I was administered by local injection to the intramyocardial/subepicardial area starting from IMA-LAD anastomosis to peripheral, either into the region of a diagonal branch or the peripheral LAD (depending on the preoperative coronary findings). IMA-LAD anastomosis was always performed; additional stenoses of the coronary vessels were supplied by an aorto-coronary venous bypass (ACVB). Postoperatively: standard protocol according to hospital procedures as for all patients with operative coronary revascularization; postoperatively, prior to discharge transthoracic echocardiography (TTE) was performed for exclusion of pericardial effusion (PE).

The first follow-up examination was planned at 12 weeks (± 1 week) postoperatively in the out-patients. A transthoracal echocardiography (TTE) an angiographic follow-up (DSA of the IMA bypass; the IMA bypasses were selectively imaged by intra-arterial digital subtraction angiography and quantitatively evaluated.)

The second follow-up examination was planned at 12 months (± 1 month) postoperatively and included TTE

Selection of Study Population

Inclusion Criteria

- Patients with coronary 1-, 2- or 3-vessel disease affecting the LAD and its lateral branches; > 50 years of age; < 80 years of age
- no past medical history of myocardial infarction;
- EF of LV: above 40%;
- cardiac catheter examination with coronary angiography: no longer than 6 months before surgery;
- revascularization of LAD by means of IMA-bypass;
- no additional/simultaneous intervention (e.g. cardiac valve, carotid artery, or other) planned or indicated.

Exclusion Criteria

- Age: < 50 years or > 80 years;
- past medical history of myocardial infarction,
- revascularization of LAD with venous bypass;
- cardiac catheter examination with coronary angiography: > 6 months preoperatively;
- EF of LV: < 40%.

Treatments

Treatment group (CABG plus FGF-I application) :
Dosing of FGF-I: 0.01 mg/kg body weight; combined injection of FGF-I with 500 IU heparin in 1 ml physiological NaCl solution and 1 ml fibrin glue (Tissucol®).

FGF-I was injected - 0.01 mg/kg body weight - into the myocardium close to the vessels after the completion of internal mammary artery (IMA) / left anterior descending coronary artery (LAD) anastomosis

Control group (CABG with application of heat-denatured FGF-I)
Dosing: application of heat-denatured FGF-I 0.001 mg/kg body weight with 500 IU heparin in 1 ml physiological NaCl solution and 1 ml fibrin glue.

Investigational Product

FGF-I was obtained from strains of Escherichia coli by genetic engineering, then isolated and highly purified. Several animal experiments proved evidence of the apathogenic action and neo-angiogenic potency of this factor. In all the animal experiments, the development of new vessels in the ischemic myocardium could be demonstrated angiographically (Circulation.1998; 97:645-650).

The investigational medicinal product was produced in the laboratory of Prof. Stegmann (Dept. of Thoracic & Cardiovascular Surgery, Fulda Medical Center) in co-operation with the laboratory of surgical research, Freiburg University. Fibrin glue (Tissucol®) was commercially available.